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# GC-MS Analysis For Identification Of Bioactive Compounds In Crude Methanolic Extracts Of Leaves Of *Origanum Majorana* L. Plant

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**Abstract**

The present study was aimed to do GC MS analysis to identify compounds of pharmacological importance in leaves of Origanum majorana L. (sweet marjoram). Freshly collected healthy looking leaves of the plant were analysed for presenc eof various compounds by preparing methanolic extract and analysed through ultra GC MS chromatograph mass spectrophotometer. Further the spectra was analysed for the characterization of specific compound it belonged to present study has investigated gc-ms profiling of crude methanolic-extract of leaves and found 21 compounds in it. Many compunds of medicinal importance including phytols, squalene, linalool and tau-cadinol have been identified in the present study. The leaf extract was dissolved in solvent methanol and analysed for presence of different compounds. The attributes in GC-MS graph viz. peak area, and retention time were used to identify and deduce molecular formulae of thr compound from WILEY8.LIB and NIST11.LIB of Schimadzu GCMS-QP2010 Ultra. Some of these compounds are being used in treatments of human diseases and some are of industrial and economic importance.

***Keywords:*** *GC-MS analysis. Linalool,* Origanum marjorana, *Phytols, Squalene.*

## 1. Introduction

Origanum majorana L. is an important member of family Lamiaceae. It is commonly called as sweet marjoram by local people. Since decades this plant has been used as an integral part of herbs and drugs to treat various diseases. This plant is native to Mediterranean region but due to it medicinal properties it has been cultivated all over the world but mainly in countries of Asia, North Africa, and Europe. Forefathers of human societies in different areas of earth are well known to its potential and utilizing it in many ways to compliment diet regularly.

Many pharmacological properties as a potent drug are the important feature of this plant such as as antioxidative, antiinflammatory, cardioprotective , hepatoprotective activity and regulation of menstrual cycle etc (Ramadan, et al. 2013, Nakatani, et al. 2000, Ryszard, et al. 2009, Vallverdu, et al. 2015, Erenler, et al. 2016). Along with these activities it is well known and used as antimicrobial agent for treating many diseases. These pharmacognostic properties are based on the various pharmacological compounds found in it. Essential oil obtained from its areal part has been claimed to have various phytols, phenolics and other compounds of clinical importance. Its leaves are also used as a beneficial antimicrobial drug in dried form or as an extract or essential oil. Essential oil of leaves of sweet marjoram has been used to treat infections of respiratory and gastrointestinal tract (Aghili, et al. 2009). Conventionally, the leaves of sweet marjoram have been used to treat and cure diabetes, insomnia, catarrh, asthma and nervousness (Cano and Volpato, 2004). It provides good amount of vitamins viz. vitamin A and vitamin C so used as garnishing in salads and vegetables. Essential oil made from its leaves is traditionally used as cure for anxiety, ulcers, fungal, bacterial and protozoan infections (Baatour, et al, 2011). To-date, many studies have been conducted for characterization of various biologically important compounds present in essential oil and different extracts of sweet marjoram leaves but no published data has been found to reveal compounds present in methanolic extract Present investigation was aimed to explore and recognize compounds present in methanolic-extract of leaves through GC-MS so that further medicinal application of it can be enhanced for human welfare.

## 2. Materials and Methods

2.1 Sample collection: Healthy looking fresh leaves of O. *majorana* were identified and collected from jaipur, Rajasthan state. Leaves were thoroughly washed in running water for removal of any dust particles stuck. Further, air drying was performed and these leaves were packed in paper bags and stored in moisture free containers.

2.2 GC-MS Analysis: GC-MS analysis was done at "Jawaharlal Nehru University, Delhi". The GC-MS analysis of plant extract was performed by GCMS-QP2010 Ultra gas chromatograph mass spectrometer.

2.3 Sample preparation: The dried leaves were powdered and a mixture was prepared in methanol solvent. For this 5 g of sample was dissolved in 20 ml methanol. Whatmann filter 1 was used for filtertaion of this dilution which was transferred in glass vials for GC-MS.

2.4 GC Method

Following method and instruments parameters were set to GC and MS for profiling of marjoram plant extract.

| GC Programme | |
|---|---|
| Ion Source Temp. | 230.00 °C |
| Interface Temp. | 270.00 °C |
| Solvent Cut Time | 5.50 min. |
| Detector Gain Mode | Relative |
| Detector Gain | +0.00 kV |
| Threshold | 1000 |
| MS Table | |
| Start Time | 6.00 min. |
| End Time | 49.98 min. |
| ACQ Mode | Scan |
| Event Time | 0.20 sec. |
| Scan Speed | 3333 |
| Start m/z | 40.00 |
| End m/z | 650.00 |

## 3. Results and Discussion

In GC-MS profile (fig.1 and table 1) of O. *majorana* leaf extract 21 compounds were identified and classified. The phytochemicals dissolved in solvent were identified on the basis of their respective attributes in GC-MS graph (peak area, retention time) and molecular formulae were confirmed from WILEY8.LIB and NIST11.LIB of Schimadzu GCMS-QP2010 Ultra. The major compounds found in the extracts of sweet marjoram leaf parts from GC-MS analysis revealed by GC chromatogram are summarised in table 1. 5. In the present study 1,6-Octadien-3-Ol, 3,7-Dimethyl (Linalool), Bicyclo[3.1.1]Hept-2-Ene, 2,6-Dimethyl-6-(4-Met), Tau.-Cadinol, Phytol, Silane, [(3,7,11,15-Tetramethyl-2-Hexadecenyl)Oxy] Trimethyl and Squalene have been identified as the major compounds in methanolic extract of leaves of O. *majorana*. Leaves of sweet marjoram have been traditionally used as antimicrobial agent, antiseptic drug, antidote, carminative and antitussive use. Similarly it has been widely used as a cure for gastrointestinal disorder, head cool. In some areas it is conventionally used as treatment for unilateral facial paralysis, headache and epilepsy. It has also been used to treat various medical conditions such as cataracts, near and far sightedness, pain and infections of ear, as well as shortness of breath, cardiac –pain, abnormality in the physiological rhythm of heart and cramps. It has been reported that water -extract, essential -oil, and ethyl- acetate extracts of aerial part of O. *majorana* exhibit major antioxidant activity against many UTI bacteria Erenler, et al. 2016; Mossa and Nawwar, et al. 2011; Hussain, et al., 2011; Triantaphyllou, et al., 2001; Chrpova, et al., 2010). Various studies have also proved presence of linalyl acetate, terpineol, thymol, Linalool and carvacrol in essential oil extracted from leaves of O. *Majorana* (Hussain, et al., 2011; Ramos, et al., 2011; Charai, et al., 1996). In other studies also various organic (phenolic) acids mainly gallic-acid, caffeic-acid, Vanillic-acid, syringic-acid, p- and m-Hydroxybenzoic-acid, cryptochlorogenic -acid and caftaric-acid are reported from extracts of leaves of this plant. From aqueous extract also identified compounds have shown remarkable antioxidant activity (Erenler, et al. 2016; Mossa and Nawwar, et al. 2011; Hussain, et al., 2011; Triantaphyllou, et al., 2001; Chrpova, et al., 2010). Squalene found in this study is a triterpene recognized as a polyunsaturated hydrocarbon; an intermediate of the biosynthetic pathway of cholesterol. Naturally it is found in liver oil of shark, olive oil and human sebum. Studies have proved effective inhibitory effects of squalene on chemically induced tumors in rodents. It has also been established in an independent experiment that it exhibits chemoprotective effect during cancer treatments by mechanism of farnesylation of Ras protein, intonation of carcinogen activation and anti-oxidative activities. However, long-term effects of taking more amount of squalene are still unknown (Theresa, 2005). It has been used as a potent cosmetic agent for making dermatologicals such as skin hydrating and softening lotions lotions (Kim and Karadeniz, 2012; Reddy and Courveur, 2009). Another compound 3, 7-dimethyl-1, 6-octadien-3-ol which is commonly recognized as linalool is also found in present study from GC-MS of methanolic extract of leaves of sweet marjoram. It is also a pharmacologically and industrially important chemical as it is used in manufacturing of pesticides

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against insects which are big threat to crops, food products, or textile fabrics (Annonymous a, 2018; Baatour, 2011). It is also used as an important ingredient in production of air care products, Laundry and dishwashing products, Cleaning and furnishing care and aroma products (Annonymous a, 2018; Prerna and Vasudeva, 2015; Young, et al., 2009). Other important compound characterized in this study is Tau.-Cadinol also known as epi-alpha-cadinol. It has also been isolated from aerial parts of *Baccharoides lilacina* as an important component (Lima, et al., 2016). Similarly it has been isolated and identified through GC-MS analysis form *Onychopetalum amazonicum* and found to be effective against numerous UTI bacteria. Same compound has been isolated from essential oil of *Senecio nudicaulis* Wall., a hilly plant of India. This oil has proved to possess antioxidant activity by various scavenging assays conducted for it (Sharma and Shah, 2015). Similarly it is obtained from *Munnozia senecionidis* Benth also, and identified by GC-MS (Annonymous b, 2018). In other studies also, ethanolic extract of the leaves of marjoram have showed antioxidant and free radical-scavenging activity Ryszard, et al., 2009). Phytol is also obtained and characterized from leaves of sweet majoram in this study. It is an acyclic diterpene alcohol and a major component of chlorophyll. It is well known to be as a precursor in manufacturing of synthetic variant of vitamin E and vitamin $K_1$. Phytol is also shown to control transcription .Phytol is confirmed to possess anticonvulsant activity by modulating of neurotransmitter systems (Costa, et al., 2012). Phytol is found to induce apoptosis and inhibit tumor progression factor in lung carcinoma cell line (Thakor, et al., 2017).

## 4 Table and Figure

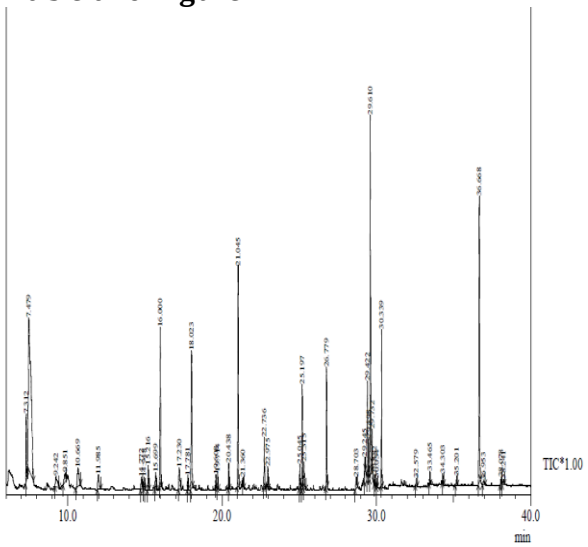


Figure 4.1: GC-MS profile of methanolic leaf extracts of *Origanum majorana* L.

Table 4.2: List of compounds isolated and identified through GC-MS analysis of methanolic leaf extracts of *Origanum majorana* L.

| S.No. | Name of compound | Retention time | Percent |
|---|---|---|---|
| 1. | 3,7-dimethyl-1,6-octadien-3-ol | 7.395 | 25.51 |
| 2. | l-proline, 1-acetyl- | 10.669 | 1.68 |
| 3. | butanoic acid, 3-hexenyl ester, (z)- | 11.985 | 1.09 |
| 4. | bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-met | 16.000 | 6.58 |
| 5. | bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8- | 17.230 | 1.42 |
| 6. | phenol, 3,5-bis(1,1-dimethylethyl)- | 18.023 | 5.85 |
| 7. | tau.-cadinol | 21.045 | 8.08 |
| 8. | 1-octanol, 3,7-dimethyl- | 22.736 | 1.52 |
| 10. | -pentadecanone, 6,10,14-trimethyl- | 25.197 | 3.51 |
| 11. | 6,10,14-trimethyl-pentadecan-2-ol | 25.315 | 1.09 |
| 12. | hexadecanoic acid, methyl ester | 26.779 | 3.54 |
| 13. | 9-octadecenoic acid (z)-, methyl ester | 29.422 | 4.08 |
| 14. | phytol | 29.610 | 10.85 |
| 15. | silane, [(3,7,11,15-tetramethyl-2-hexadecenyl)oxy]trimethyl | 30.339 | 3.56 |
| 16. | squalene | 36.668 | 9.60 |

## 5. Conclusions

From the above discussion it can be concluded that present study has found such pharmacologically and industrially important compounds from *Origanum majorana* L. leaves which are of clinical importance. This study will help further research to find efficacy and possibility of human use of these drugs as antagonist of microbial infections and as a potent aget in cancer treatment. Further it is expected that these compounds can be proved as an alternative of chemical based treatment of various diseases as a whole or as a derivative produced from these phytochemicals.

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